Accelerated Publications

Nucleotide Excision Repair of 5-Formyluracil in Vitro Is Enhanced by the Presence of Mismatched Bases†

Katsuhito Kino,‡ Yuichiro Shimizu,‡,§ Kaoru Sugasawa,‡,⊥ Hiroshi Sugiyama,¶ and Fumio Hanaoka*,‡,⊥,§

Cellular Physiology Laboratory, RIKEN and CREST, Japan Science and Technology Corporation, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan, Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan, and Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan

Received November 28, 2003; Revised Manuscript Received January 28, 2004

ABSTRACT: 5-Formyluracil (fU) is a major thymine lesion produced by reactive oxygen radicals and photosensitized oxidation. Although this residue is a potentially mutagenic lesion and is removed by several base excision repair enzymes, it is unknown whether fU is the substrate of nucleotide excision repair (NER). Here, we analyzed the binding specificity of XPC−HR23B, which initiates NER, and cell-free NER activity on fU opposite four different bases. The result of the gel mobility shift assay showed that XPC−HR23B binds the fU-containing substrates in the following order: fU:C ≫ fU:T > fU:G > fU:A. Furthermore, in the presence of XPC−HR23B, the dual incision activity was the same as the order of the binding affinity of XPC−HR23B to fU. Therefore, it is concluded that even fU, regarded as a shape mimic of thymine, can be recognized as a substrate of NER incision, and the efficiency depends on instability of the base pair.

Organisms have multiple DNA repair pathways that remove various DNA lesions, thereby maintaining the integrity of the genome and cellular functions (*1*−*5*). One such DNA repair pathway, base excision repair (BER), corrects a number of spontaneously and environmentally induced genotoxic or miscoding base lesions (*3*−*5*). Meanwhile, nucleotide excision repair (NER) is another important DNA repair system, which can eliminate a wide variety of lesions, such as UV-induced cyclobutane pyrimidine dimers and (6−4) photoproducts, as well as intrastrand cross-links and bulky adducts induced by chemical carcinogens (*1*). Impaired NER activity has been associated with several human genetic disorders, including xeroderma pigmentosum (XP). Patients suffering from XP are characterized clinically by cutaneous hypersensitivity to UV exposure and increased susceptibility to skin cancer. The gene responsible for the defect in the XP genetic complemention group C patients encodes XPC protein, which exists in vivo as a heterotrimeric complex with HR23B protein and centrin 2 (*6*, *7*). Although centrin 2, playing an important role in centrosome duplication, interacts directly with XPC, centrin 2 has little effect on the reconstituted cell-free NER reaction (*7*). Therefore, biochemical studies have revealed that the XPC−HR23B heterodimer is sufficient to specifically bind certain types of DNA lesions and initiates NER in vitro (*8*−*10*).

5-Formyluracil (fU) (Figure 1a) was identified as a novel type of oxidized thymine residue in DNA (*11*). It is a major product induced by γ-ray and UVA photosensitization with menadione (vitamin K_3), riboflavin (vitamin B_2), and nitronaphthalimide (*12*−*16*). Especially, fU can be yielded to a similar extent as 8-oxoguanine and imidazolone (*13*, *15*, *16*). Misincorporation of nucleotides opposite unrepaired fUs can induce several types of base pair substitution (T→G, T→A, and T→C) (*17*−*24*). Therefore, this product is harmful for cells (*25*). These residues in DNA opposite adenine or guanine have been shown to be removed by BER enzymes, for example, AlkA in *Escherichia coli* and hSMUG1 in human (*26*−*31*), whereas it is unknown whether fU is the substrate of NER. Here, we analyzed the binding specificity of XPC−HR23B and cell-free NER activity for fU in vitro.

MATERIALS AND METHODS

DNA Preparation. The oligonucleotide containing a single fU was prepared as described previously (*32*). The structure of 30mer containing fU, 5′-d(CTCGTCAGCA TCTfUCATCAT ACAGTCAGTG), was elucidated by enzymatic diges-

† Supported by grants from Ministry of Education, Culture, Sports, Science and Technology of Japan and a grant from the Chemical Biology Research Project of RIKEN. K.K. is a Special Postdoctoral Researcher of RIKEN.

* Corresponding author. Tel: +81-6-6879-7975. Fax: +81-6-6877-9382. E-mail: fhanaoka@fbs.osaka-u.ac.jp.

‡ RIKEN.

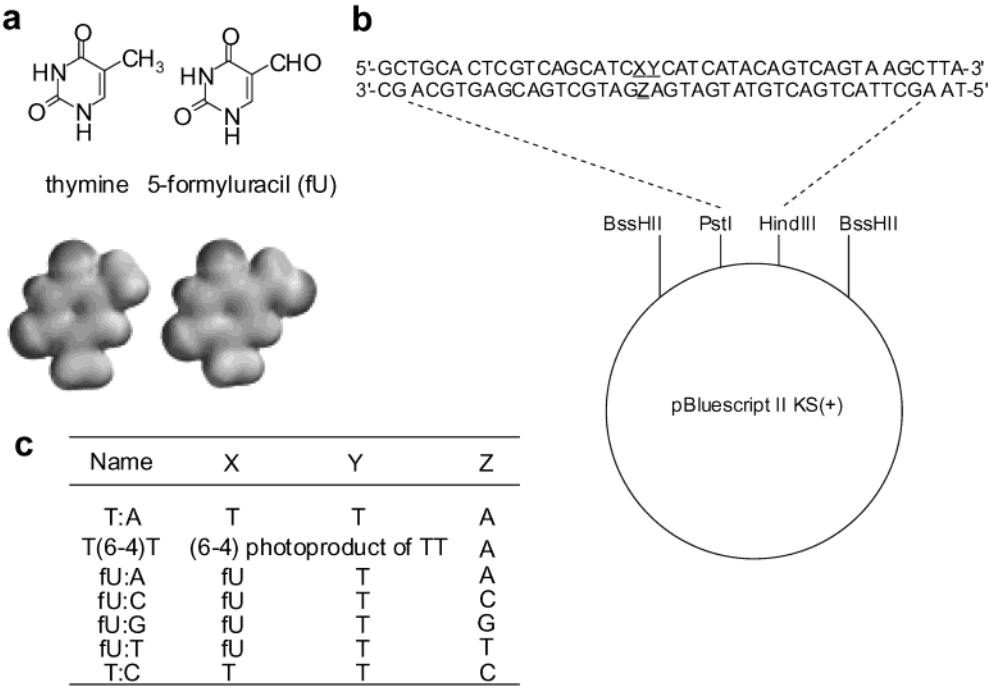
§ Osaka University.

⊥ CREST.

¶ Kyoto University.

[1] Abbreviations: fU, 5-formyluracil; XP, xeroderma pigmentosum; BER, base excision repair; NER, nucleotide excision repair; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; BSA, bovine serum albumin; AlkA, *E. coli* 3-methyladenine DNA glycosylase II; hSMUG1, human single-strand-selective monofunctional uracil-DNA glycosylase; T(6−4)T, (6−4) photoproduct of thymine dimer; DDB, the damaged DNA binding factor.

10.1021/bi0361416 CCC: $27.50
Published on Web 02/17/2004



c

Name	X	Y	Z
T:A	T	T	A
T(6-4)T	(6-4) photoproduct of TT		A
fU:A	fU	T	A
fU:C	fU	T	C
fU:G	fU	T	G
fU:T	fU	T	T
T:C	T	T	C

FIGURE 1: The structures of thymine (T) and 5-formyluracil (fU) (a, upper panel) and the electron density surfaces of *N*-methylated nucleobases (a, lower panel), (b) phagemid DNAs harboring the bottom strand sequences generated by ligating the corresponding complementary synthetic oligonucleotides to pBluescript II KS(+) digested by *Pst*I and *Hin*dIII, and (c) the nucleotides, indicated by X, Y, and Z, for each lesion.

tion and by ESI-MASS: 9126.2. Furthermore, it was confirmed that the reduction with $NaBH_4$ generated 5-hydroxymethyluracil. The oligonucleotide containing a (6−4) photoproduct of thymine dimer (T(6−4)T) was provided by Prof. S. Iwai. The ^{32}P-labeled, blunt-ended, 180 bp DNA fragments and internally ^{32}P-labeled, closed circular DNAs, which contain a site-specific fU opposite four different bases, were prepared (Figure 1b,c) (*33*).

Preparation of Proteins and Cell Extract. Purification and reconstitution of the XPC−HR23BHis heterodimer were carried out as described previously (*33*). XP3BE whole cell extract was prepared as described (*33*). Preparation and purification of His-tagged human AP endonuclease (hAPE) were carried out as described previously (*34*). The expression of His-hSMUG1 was induced with 1 mM isopropyl-β-d-thiogalactopyranoside (IPTG) at 30 °C for 4 h in the *E. coli* strain JM109 (DE3) using the pET28a vector (Novagen). The bacterial cell pellets were sonicated in buffer A [20 mM sodium phosphate (pH 7.5), 1 mM EDTA, 25 mM NaCl, 0.01% Nonidet P-40, 1 mM dithiothreitol (DTT), 0.25 mM phenylmethylsulfonyl fluoride (PMSF), and a protease inhibitor cocktail (1× Complete; Roche Diagnostics)] and centrifuged for 30 min at 100 000 × *g*. The supernatant was applied to a HiLoad SP Sepharose HP (Amersham Biosciences) column equilibrated with buffer B [20 mM sodium phosphate (pH 7.8), 10% glycerol, 0.01% Triton X-100, and 0.25 mM PMSF] containing 0.1 M NaCl, and bound proteins were eluted stepwise with buffer B containing 0.5 and 1 M NaCl. Proteins eluted with 0.5 M NaCl were loaded onto a column packed with TALON resin (Clontech) that had been equilibrated with buffer B containing 0.1 M NaCl and 5 mM imidazole. After extensive washing with the same buffer, bound proteins were eluted by increasing the imidazole concentration to 20, 100, and 250 mM. Finally, the 100 mM imidazole fraction containing His-hSMUG1 was loaded onto a Mono S PC 1.6/5 column (Amersham Biosciences) equilibrated with buffer B containing 0.1 M NaCl and eluted with buffer B containing 0.15 M NaCl.

Gel Mobility Shift Assay. The detailed method was previously described (*33*). Briefly, binding reactions (10 μL) were carried out at 30 °C for 30 min in mixtures including 20 mM sodium phosphate (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 150 mM NaCl, 1 mM DTT, 5% glycerol, 0.01% Triton X-100, BSA (1 μg), ^{32}P-labeled probe DNA (0.35 fmol), covalently closed circular plasmid DNA (1.5 ng), and the indicated amount of the recombinant XPC−HR23BHis complex. The reactions were then chilled on ice. The mixtures were directly subjected to 4% nondenaturing PAGE. Radioactivity was quantified using the BAS2500 bioimaging analyzer (Fujifilm). The gel was exposed to X-ray film (Fujifilm, RX-U) at −80 °C with an intensifying screen.

Nucleotide Excision Repair Assay. The detailed method was previously described (*33*). Briefly, the internally labeled, double-stranded circular DNA substrates (1×10^5 cpm, ∼29 ng) were incubated at 30 °C for 1 h in 25 μL reactions containing 40 mM Hepes-KOH (pH 7.8), 5 mM $MgCl_2$, 0.5 mM DTT, 70 mM NaCl, 6.6% glycerol, 0.5 mM EDTA, 2 mM ATP, and 20 μM each of dATP, dGTP, and dTTP, 8 μM dCTP, 22.5 mM creatine phosphate (di-Tris), phosphocreatine kinase (1.25 μg), BSA (9 μg), the XP3BE whole cell extract (100 μg protein), and the indicated amount of the XPC−HR23BHis complex. The reactions were stopped by addition of EDTA to a final concentration of 10 mM, and DNA was purified and subjected to 10% denaturing PAGE followed by autoradiography.

Nicking Assay. The fU-strand oligonucleotide, 5′−d(CTCGTCAGCA TCTfUCATCAT ACAGTCAGTG), was 5′ end-labeled by treatment with T4 polynucleotide kinase and [γ-^{32}P]ATP. After DNA was purified, each oligonucleotide, 5′-d(CACTGACTGT ATGATGXAGA TGCTGAC-

GAG) (X = A, G, T, and C), was added and annealed by heating at 65 °C for 10 min and then gradually cooling to 16 °C. The nicking reactions were performed in mixtures (25 μL) containing 25 mM Hepes-KOH, pH 7.8, 0.5 mM EDTA, 50 mM NaCl, 2 mM $MgCl_2$, 0.5 mM DTT, BSA (9 μg), and the substrate DNA (40 fmol). His-hSMUG1 (100 pg) and His-hAPE (2 ng) were added and incubated at 30 °C for 1 h. Reactions were stopped by adding an equal volume of stop buffer (50 mM Tris-HCl, pH 7.5, 25 mM EDTA, 0.3 mg/mL yeast tRNA, 2% SDS, and 0.8 mg/mL proteinase K) and incubated further at 30 °C for 30 min. The DNA was purified by phenol/chloroform extraction and ethanol precipitation and subjected to 16% denaturing PAGE followed by autoradiography.

RESULTS AND DISCUSSION

Gel Mobility Shift Assay for the Binding Affinity of XPC−HR23B. To examine the damage-binding specificity, a convenient, highly sensitive assay was developed by use of the gel mobility shift technique (*33*). Blunt-ended, radiolabeled DNA fragments (180 bp in length) containing a single lesion at a defined position were used for binding reactions with the purified recombinant human XPC−HR23B complex. Since centrin 2 does not greatly affect either damage binding or the NER reaction (*8*−*10*), we used the XPC−HR23B heterodimer for the following experiments. When XPC−HR23B was incubated with the ^{32}P-labeled nondamaged probe (negative control), little amount of shift band was observed (Figure 2a, lane 4). Using the probe containing T(6−4)T (positive control), we demonstrated that XPC−HR23B is capable of binding T(6−4)T in a highly specific manner (Figure 2a, lanes 6−8). These results were consistent with the previous data (*33*).

When fU was present opposite adenine, binding of XPC−HR23B was as low as the case of a normal T:A pair (Figure 2a, lanes 4 and 12). 5-Formyluracil residue, one of oxidative products of thymine, could be regarded as a shape mimic of thymine and has the same hydrogen accessibility, as shown in Figure 1a. Therefore, the fU residue likely forms the canonical Watson−Crick pair with the opposite adenine residue (*35*, *36*), which can explain why XPC−HR23B hardly recognizes fU:A.

Intriguingly, the gel mobility shift assay revealed that XPC−HR23B binds fU:C with high affinity, which is comparable to that for T(6−4)T (Figure 2a, lanes 6−8 and 14−16). Furthermore, the binding affinity of XPC−HR23B for fU:G and fU:T was moderate, but not as high as that for fU:C (Figure 2a, lanes 18−20 and 22−24). The percentages of the labeled DNA probes bound to XPC−HR23B were calculated and plotted in Figure 2b, showing that binding affinity of XPC−HR23B can be varied by opposite bases in the following order: T(6−4)T ≈ fU:C ≫ fU:T > fU:G > fU:A ≈ T:A. It should be noted that this order of the affinity correlates with the melting temperature of fU-containing oligonucleotide duplex described previously: fU:A > fU:G > fU:T > fU:C (*30*). Given that presence of unpaired bases is crucial for recognition by XPC−HR23B (*8*, *10*, *33*), this order seems reasonable.

NER Assay for the Dual Incision of fU. As XPC−HR23B can thus recognize the fU residue depending on the opposite base, cell-free NER activity on fU opposite four different

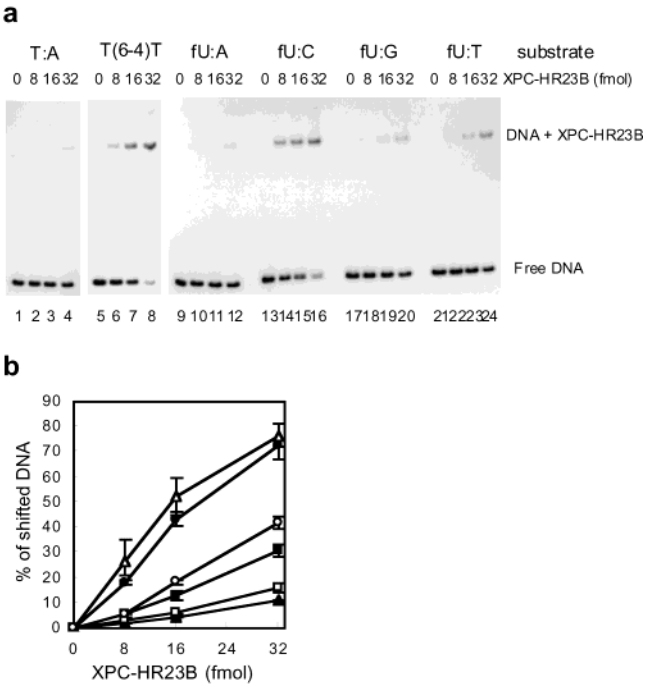


FIGURE 2: Gel mobility shift analysis of XPC−HR23B binding to fU-containing substrate opposite A, C, G, or T. In panel a, the ^{32}P-labeled DNA substrate (180 bp fragment) containing fU opposite four different bases, a normal T:A pair, or (6−4) photoproduct of thymine dimer at the corresponding position (0.35 fmol each) were incubated at 30 °C for 30 min with the indicated concentrations of XPC−HR23B. The resulting DNA-protein complex was resolved by native PAGE. In panel b, the percentage of the ^{32}P-labeled DNA substrate bound to XPC−HR23B was calculated for each lane in panel a: (▲) T:A; (△) T(6−4)T; (□) fU:A; (■) fU:G; (○) fU:T; (●) fU:C. The mean values and standard errors were calculated from at least two independent experiments.

bases was then investigated. The fU-containing synthetic oligonucleotide was ^{32}P-labeled at the 5′-end and used for synthesis of covalently closed circular DNAs, which contain an internal ^{32}P-label at 12 nucleotides 5′ to the fU lesion (*33*). Cell-free NER reactions were performed using the whole cell extract lacking the XPC protein supplemented with various amounts of purified XPC−HR23B. In the absence of XPC−HR23B, the NER reaction did not occur at all with any substrates (Figure 3a, lanes 1, 5, 9, 13, 17, and 21). In addition, when the internally labeled nondamaged substrate (negative control) was incubated in the presence of XPC−HR23B, dual incision products were below detectable level (Figure 3a, lanes 2−4), although it was reported that NER incision could occur on nondamaged DNA, which was nonetheless ∼100 times less efficient than T(6−4)T (*37*). However, using the T(6−4)T substrate (positive control), dual incision products could be clearly detected in our system (Figure 3a, lanes 22−24).

When the ^{32}P-labeled fU:C substrates were incubated with the XPC whole cell extract and XPC−HR23B, significant amounts of dual incision products could be observed (Figure 3a, lanes 10−12). However, the efficiency was much lower than that for T(6−4)T, although XPC−HR23B appeared to recognize fU:C as well as T(6−4)T (Figure 2b). Furthermore, dual incision products of fU:T and fU:G substrates could be also detected (Figure 3a, lanes 14−16 and 18−20), whereas with the fU:A substrate, the incision efficiency was almost at the background level (Figure 3a, lanes 6−8). Quantification of the incision products was summarized in Figure 3b.

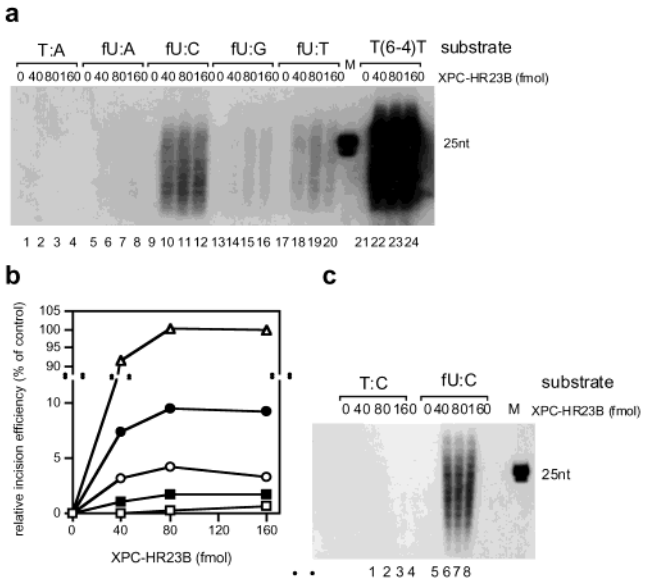


FIGURE 3: Presence of mismatched bases opposite fU enhances the NER incision. In panel a, the indicated internally labeled substrates were assayed for NER incision in the XPC whole cell extract supplemented with various concentrations of XPC−HR23B. A part of the autoradiograph showing the dual incision products is presented. "M" is ^{32}P-labeled 25-bp ladder. In panel b, the percentage of the incision products in the ^{32}P-labeled DNA substrate was calculated for each lane in panel a: (△) T(6−4)T; (□) fU:A; (■) fU:G; (○) fU:T; (●) fU:C. The quantity of the T(6−4)T incision products with XPC−HR23B (160 fmol) was set as 100%. Panel c shows the NER assay with the T:C substrate. The indicated internally labeled substrates were assayed for NER incision in the XPC whole cell extract supplemented with various amounts of XPC−HR23B. "M" is ^{32}P-labeled 25-bp ladder.

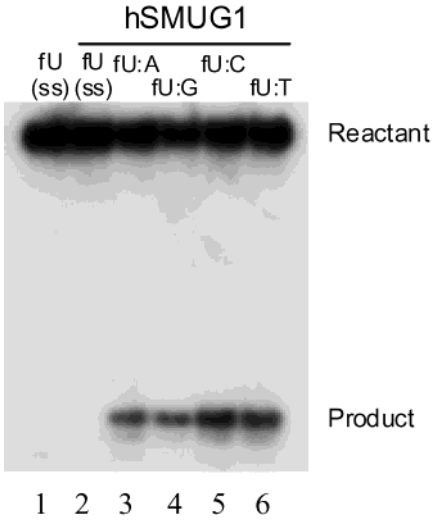


FIGURE 4: The BER incision of fU-containing DNA by hSMUG1. The ^{32}P-labeled fU-containing ss-30mer (40 fmol) and ds-30mer opposite four different bases were incubated at 30 °C for 60 min with hSMUG1 (3.0 fmol) and hAPE (53 fmol).

In the presence of XPC−HR23B, NER incision efficiency of the substrates containing fU depended on opposite bases in the following order: fU:C ≫ fU:T > fU:G ≫ fU:A, which is the same as the order of the binding affinity of XPC−HR23B to fU. These results indicate that the NER efficiency may be determined, at least partly, by the binding efficiency of XPC−HR23B.

One possible explanation for the low incision efficiency on fU is that the fU lesions may be rapidly removed by other repair pathways, such as BER, in the cell extract used and consequently only very few were processed by NER. To test this possibility, the DNA substrates that were purified from the NER reactions were treated with hSMUG1, which can excise the fU residue (*30*, *31* and Figure 4). The results indicate that more than 90% of fU:A and about 70% of fU:G, fU:T and fU:C still remained in the substrates (data not shown). Therefore, the data in Figure 3 more or less reflect the NER efficiency on the fU substrates.

It was previously shown that XPC−HR23B strongly binds the bubble-like structure without any damaged bases, although the NER incision does not occur at all with those substrates (*33*). Consistently, the NER incision on the T:C mismatch substrate was only at the background level, confirming that the incision on fU:C was significant (Figure 3c). These results demonstrate that, while instability of base pairs is important for the initial recognition by XPC−HR23B, the presence of damaged bases must be verified thereafter to induce NER incision, which requires completely different structural features associated with lesions. Therefore, low incision efficiency of fU:C could be because the damage verification activity may not work well for fU. An alternative explanation for the low incision efficiency of fU:C could be made by considering involvement of the damaged DNA binding factor (DDB). DDB has been shown to specifically bind to various types of lesions and stimulate NER in vivo (*38*, *39*) as well as in vitro (*40*, *41*). Although the stimulatory effect is more evident with cyclobutane-type pyrimidine dimer, DDB could also enhance cell-free NER incision of T(6−4)T. Although it remains to be examined whether DDB can recognize fU, it is possible that DDB, which may be present in the cell extract used for our NER reactions, may stimulate incision of T(6−4)T, but not that of fU.

Implications. We conclude that even fU, regarded as a shape mimic of thymine, can be somewhat recognized as a substrate of NER and the efficiency depends on instability of the base pair. In other words, although the NER incision system is necessary for repair of the bulky damage sites where normal base pairing is disturbed (*42*−*44*), NER enzymes unfortunately happen to incise fU opposite misincorporated nucleotides and thus accelerate fixation of point mutations. This mutagenic subreaction was also observed with several BER enzymes against fU (*28*, *29*, *31*). Furthermore, the presence of mismatched base enhances the NER efficiency of the substrates containing cisplatin adducts (*45*) and cyclobutan-type pyrimidine dimer (*33*, *46*). It has been shown that guanine (*18*, *21*, *24*) and cytosine (*17*, *19*) could be incorporated opposite fU in vitro. In *E. coli*, fU could induce T→C and T→A mutations (*20*), whereas the data in other experiments indicated that fU:G, fU:T, and fU:C could be formed during DNA replication (*22*). Furthermore, in mammalian cells, T→G and T→A mutations could be induced by fU (*23*). Thus mutations caused by fU could differ depending on the experimental systems used. We also observed that human DNA polymerase η promotes misincorporation of three nucleotides; guanine was incorporated more efficiently than cytosine and thymine opposite fU (Kino, K., Ito, N., Hanaoka, F. Unpublished). Therefore, fU opposite misincorporated nucleotide may occur quite frequently, and NER incision of such lesions can be harmful as well as mutagenic BER subreaction (*28*, *29*, *31*) and NER incision of other mismatch substrates (*33*, *45*, *46*). As MutY

and hMYH incise adenine paired with 8-oxoguanine (*47*), unknown enzymes that directly incise the misincorporated bases opposite fU could exist in cells. Thus, these systems should inhibit the mutagenic repair and are very crucial for keeping genetic codes.

ACKNOWLEDGMENT

We are grateful to all the members of Cellular Physiology Laboratory for helpful discussions and encouragement. We also thank Prof. S. Iwai for providing the (6−4) photoproduct of thymine dimer oligonucleotide.

REFERENCES

1. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, ASM Press: Washington, DC.
2. Hoeijmakers, J. H. J. (2001) Genome maintenance mechanisms for preventing cancer, *Nature 411*, 366−374.
3. McCullough, A. K., Dodson, M. L., and Lloyd, R. S. (1999) Initiation of base excision repair: glycosylase mechanisms and structures, *Annu. Rev. Biochem. 68*, 255−285.
4. Krokan, H. E., Nilsen, H., Skorpen, F., Otterlei, M., and Slupphaug, G. (2000) Base excision repair of DNA in mammalian cells, *FEBS Lett. 476*, 73−77.
5. Nilsen, H., and Krokan, H. E. (2001) Base excision repair in a network of defence and tolerance, *Carcinogenesis 22*, 987−998.
6. Masutani, C., Sugasawa, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., Takio, K., Tanaka, K., van der Spek, P. J., Bootsma, D., Hoeijmakers, J. H. J., and Hanaoka, F. (1994) Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homologue of yeast RAD23, *EMBO J. 13*, 1831−1843.
7. Araki, M., Masutani, C., Takemura, M., Uchida, A., Sugasawa, K., Kondoh, J., Ohkuma, Y., and Hanaoka, F. (2001) Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair, *J. Biol. Chem. 276*, 18665−18672.
8. Sugasawa, K., Ng, J. M. Y., Masutani, C., Iwai, S., van der Spek, P. J., Eker, A. P. M., Hanaoka, F., Bootsma, D., and Hoeijmakers, J. H. J. (1998) Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair, *Mol. Cell 2*, 223−232.
9. Batty, D., Rapic'-Otrin, V., Levine, A. S., and Wood, R. D. (2000) Stable binding of human XPC complex to irradiated DNA confers strong discrimination for damaged sites, *J. Mol. Biol. 300*, 275−290.
10. Kusumoto, R., Masutani, C., Sugasawa, K., Iwai, S., Araki, M., Uchida, A., Mizukoshi, T., and Hanaoka, F. (2001) Diversity of the damage recognition step in the global genomic nucleotide excision repair in vitro, *Mutat. Res. 485*, 219−227.
11. Kasai, H., Iida, A., Yamaizumi, Z., Nishimura, S., and Tanooka, H. (1990) 5-Formyldeoxyuridine: a new type of DNA damage induced by ionizing radiation and its mutagenicity to Salmonella strain TA102, *Mutat. Res. 243*, 249−253.
12. Bjelland, S., Eide, L., Time, R. W., Stote, R., Eftedal, I., Volden, G., and Seeberg, E. (1995) Oxidation of thymine to 5-formyluracil in DNA: mechanisms of formation, structural implications, and base excision by human cell free extracts, *Biochemistry 34*, 14758−14764.
13. Douki, T., Delatour, T., Paganon, F., and Cadet, J. (1996) Measurement of oxidative damage at pyrimidine bases in gamma-irradiated DNA, *Chem. Res. Toxicol. 9*, 1145−1151.
14. Saito, I., Takayama, M., and Kawanishi, S. (1995) Photoactivatable DNA-cleaving amino acids highly sequence selective DNA photocleavage by novel L-lysine derivatives, *J. Am. Chem. Soc. 117*, 5590−5591.
15. Wang, Y., Men, L., and Vivekananda, S. (2002) Fragmentation of deprotonated ions of oligodeoxynucleotides carrying a 5-formyluracil or 2-aminoimidazolone, *J. Am. Soc. Mass Spectrom. 13*, 1190−1194.
16. Frelon, S., Douki, T., Favier, A., and Cadet, J. (2003) Hydroxy radical is not the main reactive species involved in the degradation of DNA bases by copper in the presence of hydrogen peroxide, *Chem. Res. Toxicol. 16*, 191−197.
17. Zhang, Q.-M., Sugiyama, H., Miyabe, I., Matsuda, S., Saito, I., and Yonei, S. (1997) Replication of DNA templates containing 5-formyluracil, a major oxidative lesion of thymine in DNA, *Nucleic Acids Res. 25*, 3969−3973.
18. Yoshida, M., Makino, K., Morita, H., Terao, H., Ohyama, Y., and Ide, H. (1997) Substrate and mispairing properties of 5-formyl-2′-deoxyuridine 5′-triphosphate assessed by in vitro DNA polymerase reactions, *Nucleic Acids Res. 25*, 1570−1577.
19. Zhang, Q.-M., Sugiyama, H., Miyabe, I., Matsuda, S., Kino, K., Saito, I., and Yonei, S. (1999) Replication in vitro and cleavage by restriction endonuclease of 5-formyluracil- and 5-hydroxymethyluracil-containing oligonucleotides, *Int. J. Radiat. Biol. 75*, 59−65.
20. Miyabe, I., Zhang, Q.-M., Sugiyama, H., Kino, K., and Yonei, S. (2001) Mutagenic effects of 5-formyluracil on a plasmid vector during replication in *Escherichia coli*, *Int. J. Radiat. Biol. 77*, 53−58.
21. Masaoka, A., Terao, H., Kobayashi, M., Ohyama, Y., and Ide, H. (2001) Oxidation of thymine to 5-formyluracil in DNA promotes misincorporation of dGTP and subsequent elongation of a mismatched primer terminus by DNA polymerase, *J. Biol. Chem. 276*, 16501−16510.
22. Ånensen, H., Provan, F., Lian, A. T., Reinertsen, S.-H. H. S., Ueno, Y., Matsuda, A., Seeberg, E., and Bjelland, S. (2001) Mutations induced by 5-formyl-2′-deoxyuridine in *Escherichia coli* include base substitutions that can arise from mispairs of 5-formyluracil with guanine, cytosine and thymine, *Mutat. Res. 476*, 99−107.
23. Kamiya, H., Murata-Kamiya, N., Karino, N., Ueno, Y., Matsuda, A., and Kasai, H. (2002) Induction of T→G and T→A transversions by 5-formyluracil in mammalian cells, *Mutat. Res. 513*, 213−222.
24. Miyashita, T., Ono, A., and Izuta, S. (2002) Kinetic analysis of nucleotides incorporated opposite oxidized thymine bases on template DNA, *Nucleic Acids Res. Suppl. 2*, 255−256.
25. Klungland, A., Paulsen, R., Rolseth, V., Yamada, Y., Ueno, Y., Wiik, P., Matsuda, A., Seeberg, E., and Bjelland, S. (2001) 5-Formyluracil and its nucleoside derivatives confer toxicity and mutagenicity to mammalian cells by interfering with normal RNA and DNA metabolism, *Toxicol. Lett. 119*, 71−78.
26. Zhang, Q.-M., Miyabe, I., Matsumoto, Y., Kino, K., Sugiyama, H., and Yonei, S. (2000) Identification of repair enzymes for 5-formyluracil in DNA, *J. Biol. Chem. 275*, 35471−35477.
27. Masaoka, A., Terato, H., Kobayashi, M., Honsho, A., Ohyama, Y., and Ide, H. (1999) Enzymatic repair of 5-formyluracil. I. Excision of 5-formyluracil site-specifically incorporated into oligonucleotide substrates by AlkA protein (*Escherichia coli* 3-methyladenine DNA glycosylase II), *J. Biol. Chem. 274*, 25136−25143.
28. Terato, H., Masaoka, A., Kobayashi, M., Fukushima, S., Ohyama, Y., Yoshida, M., and Ide, H. (1999) Enzymatic repair of 5-formyluracil. II. Mismatch formation between 5-formyluracil and guanine during DNA replication and its recognition by two proteins involved in base excision repair (AlkA) and mismatch repair (MutS), *J. Biol. Chem. 274*, 25144−25150.
29. Liu, P., Burdzy, A., and Sowers, L. C. (2003) Repair of the mutagenic DNA oxidation product, 5-formyluracil, *DNA Repair 2*, 199−210.
30. Matsubara, M., Masaoka, A., Tanaka, T., Miyano, T., Kato, N., Terato, H., Ohyama, Y., Iwai, S., and Ide, H. (2003) Mammalian 5-formyluracil-DNA glycosylase. 1. Identification and characterization of a novel activity that releases 5-formyluracil from DNA, *Biochemistry 42*, 4993−5002.
31. Masaoka, A., Matsubara, M., Hasegawa, R., Tanaka, T., Kurisu, S., Terato, H., Ohyama, Y., Karino, N., Matsuda, A., and Ide, H. (2003) Mammalian 5-formyluracil-DNA glycosylase. 2. Role of SMUG1 uracil-DNA glycosylase in repair of 5-formyluracil and other oxidized and deaminated base lesions, *Biochemistry 42*, 5003−5012.
32. Sugiyama, H., Matsuda, S., Kino, K., Zhang, Q.-M., Yonei, S., and Saito, I. (1996) New synthetic method of 5-formyluracil-containing oligonucleotides and their melting behavior, *Tetrahedron Lett. 37*, 9067−9070.
33. Sugasawa, K., Okamoto, T., Shimizu, Y., Masutani, C., Iwai, S., and Hanaoka, F. (2001) A multistep damage recognition mechanism for global genomic nucleotide excision repair, *Genes Dev. 15*, 507−521.

34. Shimizu, Y., Iwai, S., Hanaoka, F., and Sugasawa, K. (2003) Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase, *EMBO J. 22*, 164−173.
35. Tsunoda, M., Karino, N., Ueno, Y., Matsuda, A., and Takenaka, A. (2001) Crystallization and preliminary X-ray analysis of a DNA dodecamer containing 2′-deoxy-5-formyluridine; what is the role of magnesium cation in crystallization of Dickerson-type DNA dodecamers? *Acta Crystallogr. D57*, 345−348.
36. Tsunoda, M., Kondo, J., Karino, N., Ueno, Y., Matsuda, A., and Takenaka, A. (2002) Water mediated Dickerson-Drew-type crystal of DNA dodecamer containing 2′-deoxy-5-formyluridine, *Biophys. Chem. 95*, 227−233.
37. Branum, M. E., Reardon, J. T., and Sancar A. (2001) DNA repair excision nuclease attacks undamaged DNA, *J. Biol. Chem. 276*, 25421−25426.
38. Tang, J. Y., Hwang, B. J., Ford, J. M., Hanawalt, P. C., and Chu, G. (2000) Xeroderma pigmentosum *p48* gene enhances global genomic repair and suppresses UV-induced mutagenesis, *Mol. Cell 5*, 737−744.
39. Hwang, B. J., Ford, J., Hanawalt, P. C., and Chu, G. (1999) Expression of the *p48* xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair, *Proc. Natl. Acad. Sci. U.S.A. 96*, 424−428.
40. Aboussekhra, A., Biggerstaff, M., Shivji, M., Vilpo, J., Moncollin, J., Podust, V., Protic, M., Hubscher, U., Egly, J., and Wood, R. (1995) Mammalian DNA nucleotide excision repair reconstituted with purified protein components, *Cell 80*, 859−868.
41. Wakasugi, M., Kawashima, A., Morioka, H., Linn, S., Sancar, A., Mori, T., Nikaido, O., and Matsunaga, T. (2002) DDB accumulates at DNA damage sites immediately after UV irradiation and directly stimulates nucleotide excision repair, *J. Biol. Chem. 277*, 1637−1640.
42. Mao, B., Hingerty, B. E., Broyde, S., and Patel, D. J. (1998) Solution structure of the aminofluorene [AF]-intercalated conformer of the *syn*-[AF]-C^8-dG adduct opposite dC in a DNA duplex, *Biochemistry 37*, 81−94.
43. Gelasco, A., and Lippard, S. J. (1998) NMR solution structure of a DNA dodecamer duplex containing a cis-diammineplatinum-(II) d(GpG) intrastrand cross-link, the major adduct of the anticancer drug cisplatin, *Biochemistry 37*, 9230−9239.
44. Kim, J.-K., Patel, D., and Choi, B.-S. (1995) Contrasting structural impacts induced by *cis-syn* cyclobutane dimer and (6−4) adduct in DNA duplex decamers: Implication in mutagenesis and repair activity, *Photochem. Photobiol. 62*, 44−50.
45. Moggs, J. G., Szymkowski, D. E., Yamada, M., Karran, P., and Wood, R. D. (1997) Differential human nucleotide excision repair of paired and mispaired cisplatin-DNA adducts, *Nucleic Acids Res. 25*, 480−490.
46. Mu, D., Tursun, M., Duckett, D. R., Drummond, J. T., Modrich, P., and Sancar, A. (1997) Recognition and repair of compound DNA lesions (base damage and mismatch) by human mismatch repair and excision repair systems, *Mol. Cell. Biol. 17*, 760−769.
47. David, S. S., and Williams, S. D. (1998) Chemistry of glycosylases and endonucleases involved in base-excision repair. *Chem. Rev. 98*, 1221−1261.

BI0361416